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Applicants : Jiangchun Xu et al.  
Application No. : 09/656,668  
Filed : September 7, 2000  
For : OVARIAN TUMOR SEQUENCES AND METHODS OF  
USE THEREFOR

Examiner : Monika B. Sheinberg  
Art Unit : 1631  
Docket No. : 210121.484C3  
Date : September 23, 2002

#23  
Plunkett  
10/7/02

**DECLARATION OF STEVEN P. FLING Ph.D., UNDER 37 C.F.R. § 1.132**

Box RCE  
Commissioner for Patents  
Washington, D.C. 20231

The undersigned, Dr. Steven P. Fling, hereby declares:

1. I am a Scientist at Corixa Corporation, the assignee of the subject application. The following experiments were carried out under my supervision.

2. I have reviewed the Office Action dated June 17, 2002 and the Advisory Action dated September 3, 2002, in the subject application, including the rejections under 35 U.S.C. §§ 101 and 112, and provide this Declaration for the purpose of providing more evidence to the Examiner that the SEQ ID NO:198, as described in the specification is clearly over-expressed in ovarian tumors relative to normal tissue.

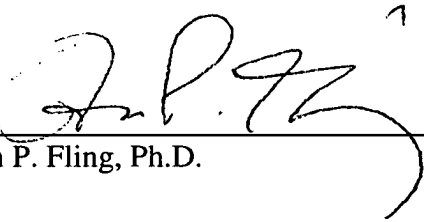
3. Expression analysis of ovarian cancer-associated sequence SEQ ID NO:198 (*i.e.*, or O590S) as performed by Real-Time PCR. Real Time PCR (*e.g.*, Gibson et al., *Genome Research* 6:995-1001, 1996; Heid et al., *Genome Research* 6:986-994, 1996) is a technique that evaluates the level of PCR product accumulation during amplification. This technique permits quantitative evaluation of mRNA levels in multiple samples. Briefly, mRNA was extracted from tumor and normal tissue and

cDNA was prepared using standard techniques. Real Time PCR was performed using a Perkin Elmer/Applied Biosystems (Foster City, CA) 7700 Prism instrument. Matching primers and fluorescent probes were designed for O590S (SEQ ID NO:198) using the primer express program provided by Perkin Elmer/Applied Biosystems (Foster City, CA). Optimal concentrations of primers and probes were determined and control (*e.g.*,  $\beta$ -actin) primers and probes were obtained commercially from Perkin Elmer/Applied Biosystems (Foster City, CA). To quantitate the amount of O590S specific mRNA in a sample, a standard curve was generated using a plasmid containing O590S. A standard curve was generated using the Ct values determined in the Real Time PCR, which were related to the initial cDNA concentration used in the assay. Standard dilutions ranging from  $10^1$ - $10^6$  copies of the gene of interest were generally sufficient. In addition, a standard curve was generated for the control sequence. This permits standardization of initial RNA content of a tissue sample to the amount of control for comparison purposes.

Real Time PCR analysis demonstrated that SEQ ID NO:198, clone 57886 or O590S, was shown to be over-expressed in over 65% of ovarian tumor samples tested, 50% of tumor samples derived from SCID mice, and 35% of ovarian tumor cell lines tested, when compared to both normal ovarian tissue, in addition to an extensive panel of normal tissue. Little or no expression was observed in normal esophagus, spinal cord, bladder, colon, liver, PBMC (activated or resting), lung, skin, small intestine, stomach, skeletal muscle, pancreas, dendritic cells, heart, spleen, bone marrow, thyroid, trachea, thymus, bronchia, cerebellum, breast, brain, bone, adrenal gland and salivary gland. Some low level expression was observed in normal kidney, ureter, uterus and peritoneum epithelium, with the expression levels in these normal tissues ranging from approximately 10-fold to 500-fold lower than the levels observed in ovary tumors over-expressing O590S (SEQ ID NO:198).

4. The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that

willful, false statements, and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

  
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Steven P. Fling, Ph.D.

Date 5/23/02

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